

60 YEARS OF NEUROENDOCRINOLOGY

The posterior pituitary, from Geoffrey Harris to our present understanding

Gareth Leng, Rafael Pineda, Nancy Sabatier and Mike LudwigCentre for Integrative Physiology, University of Edinburgh, Hugh Robson Building, George Square,
Edinburgh EH9 8XD, UKCorrespondence
should be addressed
to G Leng
Email
Gareth.Leng@ed.ac.uk**Abstract**

Geoffrey Harris pioneered our understanding of the posterior pituitary, mainly with experiments that involved the electrical stimulation of the supraoptico-hypophysial tract. In the present essay, we explain how his observations included clues to the pulsatile nature of the oxytocin signal – clues that were followed up by subsequent workers, including his students and their students. These studies ultimately led to our present understanding of the milk-ejection reflex and of the role of oxytocin in parturition. Discoveries of wide significance followed, including: the recognition of the importance of pulsatile hormone secretion; the recognition of the importance of stimulus-secretion coupling mechanisms in interpreting the patterned electrical activity of neurons; the physiological importance of peptide release in the brain; the recognition that peptide release comes substantially from dendrites and can be regulated independently of nerve terminal secretion; and the importance of dynamic morphological changes to neuronal function in the hypothalamus. All of these discoveries followed from the drive to understand the milk-ejection reflex. We also reflect on Harris's observations on vasopressin secretion, on the effects of stress, and on oxytocin secretion during sexual activity.

Key Words

- ▶ oxytocin
- ▶ vasopressin
- ▶ pituitary
- ▶ physiology

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(2015) **226**, T173–T185**Introduction**

The comfortable view of science is that it is a uniquely disinterested activity of gathering objective and unbiased observations which, by the selfless collaboration and co-operation of transnational armies of scientists, lead us ever closer to objective truth. A less comfortable view was expressed by Popper (1959): 'Science does not rest upon solid bedrock. The bold structure of its theories rises, as it were, above a swamp', and in his view, it is the 'bold ideas, unjustified anticipations and speculative thought' of individual scientists that mark the best science and that

drive progress. There is certainly a flow in scientific understanding: one observation leads to the next, and each question answered raises another. And that flow is certainly perturbed (if not quite guided) by those whose bold ideas gain currency. In the present essay, we trace the impact of the work of Geoffrey Harris on our understanding of the posterior pituitary gland, although whether our understanding would be different had Harris become an accountant instead of a scientist is something we cannot say: that is one experiment we cannot yet perform.

Harris won his reputation as the 'father of neuroendocrinology' by conducting incisive experiments which showed that the endocrine cells of the anterior pituitary are regulated by the products of hypothalamic neurons that are secreted into the hypothalamo-pituitary portal circulation (Raisman 1997). If he was bold in this, he was more conservative when it came to the theories of others: in his 1955 monograph, he still inclined to the view that the posterior pituitary contained endocrine cells that were innervated by hypothalamic neurons (Harris 1955). Although he conceded that the neurosecretory origin of the posterior pituitary hormones (Leveque & Scharrer 1953) was an 'attractive hypothesis', he declared that 'sweeping statements have been made at various times by the protagonists of the neurosecretory hypothesis' and warned that 'such claims as these, which run contrary to a great deal of established data should be taken with reserve' (Harris 1955: 264). In particular, Harris rejected the notion that the Gomorri-stainable material present in the hypothalamo-hypophysial tract was the histological representation of antidiuretic hormone, as had been argued by the Scharrers. He thought that the amount of oxytocin and antidiuretic activity present in the hypothalamus was too low to be consistent with the hypothalamus being the site of production. Finally, he disputed the evidence that neural stalk section could be followed by a partial regeneration of the neural lobe – evidence which suggested that the regeneration of nerve terminals was sufficient to support secretion in the absence of endocrine cells (Harris 1955: 262–265).

Nevertheless, Harris pioneered our understanding of the posterior pituitary, mainly with experiments that involved the electrical stimulation of the supraoptico-hypophysial tract. At the outset of those experiments, it was known that extracts of the posterior pituitary could stimulate the let-down of milk in lactating animals, and Ely & Petersen (1941) had shown that the blood of cows which had been milked contained something that could evoke milk let-down in the isolated udder. They proposed that this substance came from the posterior pituitary and was released by suckling, but Selye (1934) had earlier proposed that lactation could be explained by the stimulation of prolactin production from the anterior pituitary, and several reports had appeared that suggested that lactation could proceed normally even after sectioning the neural stalk.

Accordingly, with his student Barry Cross, Harris set out to test these two hypotheses. He had concluded (Harris 1948a) that direct electrical stimulation was ineffective in triggering secretion from the anterior pituitary, but the

posterior pituitary was innervated by a nervous tract – the supraoptico-hypophysial tract. Cross & Harris (1950, 1952) showed that the electrical stimulation of this tract caused an increase in intramammary pressure in lactating rabbits, which suggested that the pituitary contains a releasable factor that can induce milk let-down. Harris *et al.* (1969) later showed that the mammary response depended strongly on the stimulus frequency – only at frequencies in excess of 40 Hz was there an appreciable response – a finding that would prove to be prescient (Fig. 1A and B).

In 1966, Yagi *et al.* showed that electrical stimuli applied to the neural stalk triggered action potentials that were conducted antidromically to the neurosecretory cell bodies, but the utility of this seemed limited, because both the site of stimulation and the site of recording required precise stereotaxic control (Yagi *et al.* 1966). However, Cross, who was at that point Professor of Anatomy at Bristol, saw that the site of the stimulating electrode could be precisely controlled in lactating rats by ensuring that it was positioned where the stimulation would elicit a rise in intramammary pressure (Sundsten *et al.* 1970). This opened the way to studying magnocellular neurons *in vivo*, and Jon Wakerley and Dennis Lincoln, working in Cross's department, used this approach to study how the electrical activity of 'antidromically identified' magnocellular neurons regulate oxytocin and vasopressin secretion.

The milk-ejection reflex

There was still no real understanding of the milk-ejection reflex and, in particular, no appreciation that the reflex was intermittent. The breakthrough came when Wakerley & Lincoln (1973) showed that during suckling, some of the antidromically identified cells in the supraoptic and paraventricular nuclei showed brief, synchronised high-frequency discharges (about 1–2 s at 50 Hz) at intervals of about 10 min, each of which was followed about 10 s later by an abrupt increase in intramammary pressure, which is a marker of milk let-down in the mammary glands (Fig. 1D). It became clear that these bursts, which led to pulses of oxytocin secretion, were approximately synchronised among all of the magnocellular oxytocin cells in the hypothalamus. As a corollary, other magnocellular neurons that were antidromically identified as projecting to the posterior pituitary, but that did not participate in this bursting activity, could be assumed to be vasopressin cells.

Exactly why pulsatile secretion was a critically important phenomenon was not immediately apparent,

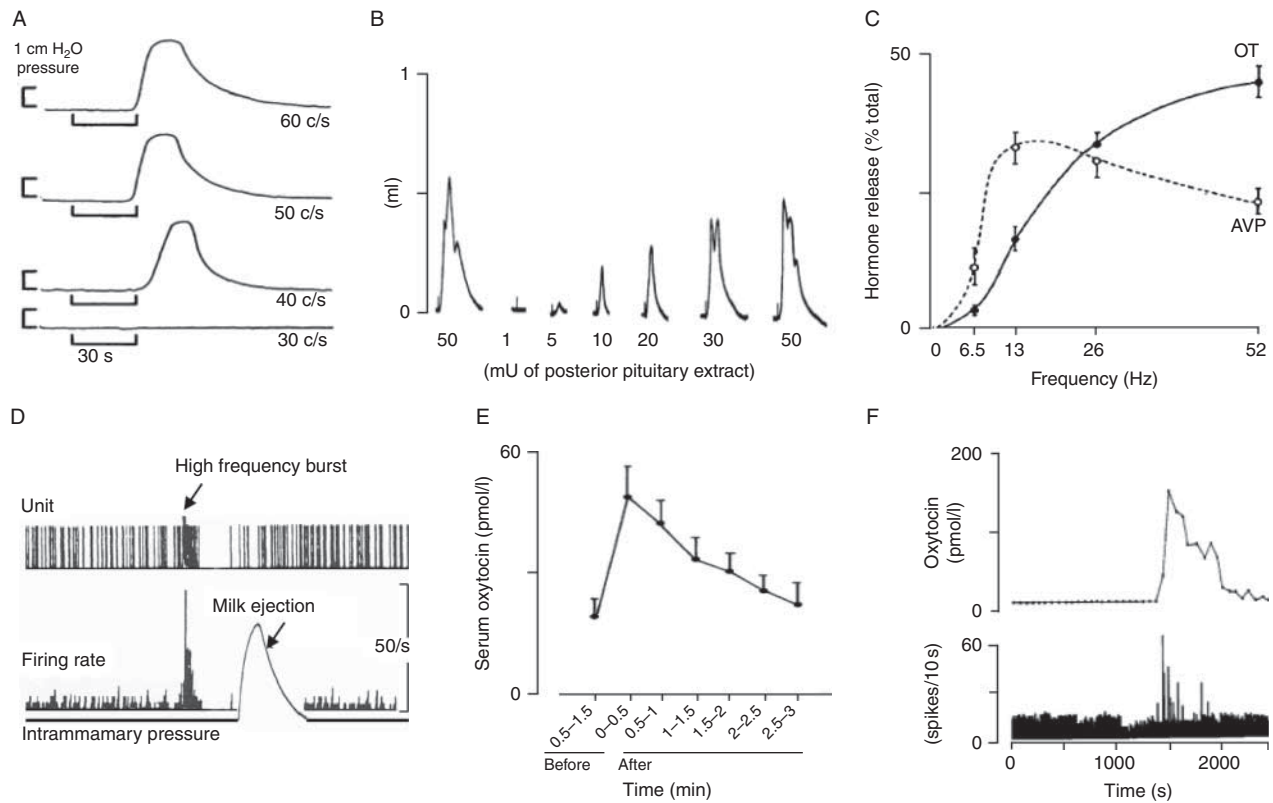


Figure 1

(A) Harris and co-workers showed that electrical stimulation of the neural stalk in lactating rabbits resulted in a sharp rise in intramammary pressure, and they inferred that this was the consequence of oxytocin secreted from the posterior pituitary. They noted that the response to stimulation depended strongly on the frequency of stimulation. The explanation for this has two components. First, the response of the mammary gland to oxytocin is non-linear. Reproduced, with permission from The Physiological Society, from Harris GW, Manabe Y & Ruf KB (1969) A study of the parameters of electrical stimulation of unmyelinated fibres in the pituitary stalk. *Journal of Physiology* **203** 67–81. Copyright 1969 The Physiological Society. (B) The rabbit mammary gland shows a threshold response to i.v. injection of 10 mU oxytocin and a near-maximal response to a dose of 50 mU. Second, the secretion of oxytocin is greatly facilitated by increasing the frequency of stimulation. Reproduced, with permission, from Cross BA & Harris GW (1952) The role of the neurohypophysis in the milk ejection reflex. *Journal of Endocrinology* **8** 148–161. (C) The amount of oxytocin (and vasopressin) that is released from the rat posterior pituitary gland *in vitro* in response to a fixed number of electrical stimulus pulses varies markedly with the frequency at which the pulses are applied (the graph plots hormone release in response to 156 pulses at each frequency).

Reproduced, with permission, from Bicknell RJ (1988) Optimizing release from peptide hormone secretory nerve terminals. *Journal of Experimental Biology* **139** 51–65. Copyright 1988 The Company of Biologists Limited. (D) During the milk-ejection reflex (MER), oxytocin neurons discharge short bursts (1–3 s) at a spike frequency that averages 40–50 spikes/s (i.e. at a frequency that optimises the efficiency of secretion and that evokes a sharp rise in intramammary pressure). Reproduced, with permission from The Physiological Society, from Lincoln DW & Wakerley JB (1974) Electrophysiological evidence for the activation of supraoptic neurones during the release of oxytocin. *Journal of Physiology* **242** 533–554. Copyright 1974 The Physiological Society. (E) This response is indeed attributable to a pulse of oxytocin, as measured in the blood by RIA. Reproduced, with permission, from Higuchi T, Honda K, Fukuoka T, Negoro H & Wakabayashi K (1985) Release of oxytocin during suckling and parturition in the rat. *Journal of Endocrinology* **105** 339–346. As shown in (F), similar bursts are observed during parturition. Reproduced, with permission, from Summerlee AJ, Paisley AC, O'Byrne KT, Fairhall KM, Robinson IC & Fletcher J (1986) Aspects of the neuronal and endocrine components of reflex milk ejection in conscious rabbits. *Journal of Endocrinology* **108** 143–149.

but an important clue lay in Harris's observation, alluded to in the previous section, that the electrical stimulation of the posterior pituitary would only evoke a strong intramammary pressure response if relatively high frequencies of stimulation were used (Harris *et al.* 1969). The explanation for this has two elements (Fig. 1). First, the response of the mammary gland to a bolus of oxytocin is non-linear and has quite a narrow dynamic range: there

is a threshold dose that must be exceeded before any effect is observed, and above this threshold, the response to higher doses of oxytocin rises swiftly to a maximum. Thus, the mammary gland seems to require pulsatile activation – especially because if oxytocin is applied continuously rather than in pulses, then the response of the gland rapidly diminishes. Second, how much oxytocin is secreted in response to electrical stimulation depends

on the frequency of stimulation – more oxytocin is secreted per stimulus pulse when the stimuli are clustered closely together (Fig. 1C). This frequency facilitation of stimulus-secretion coupling can be attributed to several factors. A solitary spike invading an axon in the pituitary will not invade all of the terminals of that axon, and in those it does invade, it will produce only a brief rise in intracellular calcium – the essential trigger for vesicle exocytosis. However, during a burst of spikes, a progressive increase in extracellular $[K^+]$ depolarises the axons and endings in the neural lobe, which thereby secures a more complete invasion of the terminal arborisation. Moreover, successive spikes in a burst are progressively broadened, which induces a progressively larger calcium entry and gives a potentiated signal for exocytosis. As a result, each spike within a burst releases much more oxytocin than that released by the isolated spikes that occur between bursts (Bourque 1991, Leng & Brown 1997).

The explosive nature of milk-ejection bursts suggested that some positive feedback was involved, so Moss *et al.* (1972) set about to try to show that oxytocin released from the posterior pituitary had that positive feedback effect. They recorded from magnocellular neurons in rats and rabbits and studied the effects of oxytocin that was administered intravenously and oxytocin that was administered directly to the neurons by iontophoresis. The results were disconcerting – oxytocin had a dramatic excitatory effect on many magnocellular neurons. This seemed to be a specific effect, because non-neurosecretory cells were unaffected and vasopressin that was applied in the same way had no effect. However, even at large doses, oxytocin had no effect at all when given intravenously.

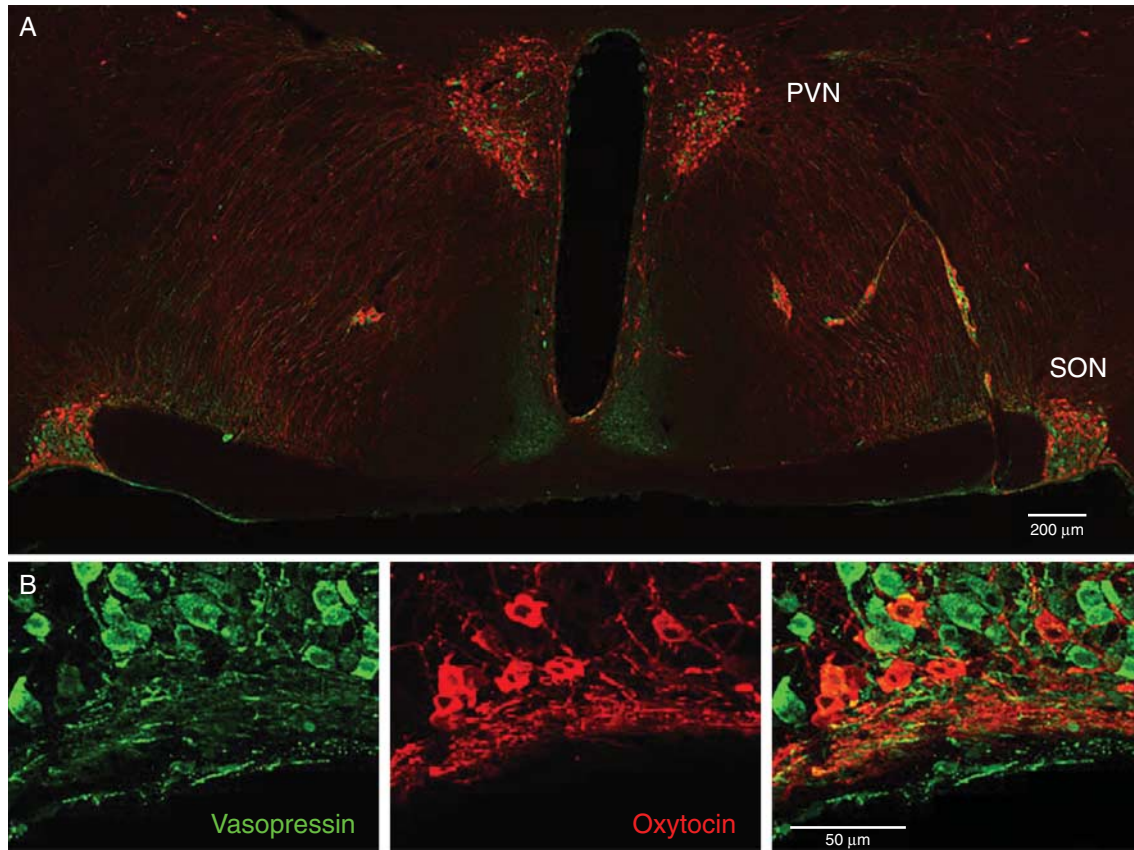
At that time, there was no evidence that oxytocin was released centrally, and indeed it seemed very unlikely that it would be. There was no strong evidence of axon collaterals, and the evidence tended to suggest that if there were any recurrent collaterals, then their effect was probably inhibitory. Several reports had appeared of 'recurrent inhibition' in the magnocellular system – reports that were later shown by Leng & Dyball (1984) to be based on misinterpreted evidence. Moss *et al.* (1972) recognised that the ineffectiveness of intravenous oxytocin meant that oxytocin secreted from the pituitary did not find its way back into the brain. Accordingly, they concluded that the excitatory action of oxytocin on oxytocin cells was a pharmacological phenomenon that had no physiological significance.

However, this view was soon to change. Richard *et al.* (1991) in France showed that oxytocin was released into

the hypothalamus during suckling, that small amounts of oxytocin injected into the brain of lactating rats dramatically facilitated the milk-ejection reflex, and that central injections of oxytocin antagonist could block the reflex. Thus, it seemed that oxytocin given centrally was somehow able to 'orchestrate' the intermittent bursting activity of oxytocin cells that had first been seen by Wakerley & Lincoln (1973). This was the first convincing demonstration of a physiological role for a peptide in the brain, and it led the way to a transformation of our understanding of information processing in the nervous system. We now know that more than one hundred different neuropeptides are expressed in different neuronal populations and that most if not all neurons in the brain release one or more peptide messengers as well as a conventional neurotransmitter. Because peptides have a relatively long half-life and act at receptors with nanomolar affinity, their actions are not confined to targets that are in direct apposition to the site of release. Importantly, peptide signals in the brain often have organisational and activational roles that seem more akin to the roles of hormones in the periphery (Ludwig & Leng 2006). We now take for granted this understanding that peptides in the brain can have specific functional roles, because of our knowledge of many peptides that evoke coherent behavioural responses when they are injected into the brain.

In Germany, Landgraf *et al.* (1992) began measuring oxytocin and vasopressin release in the brain using the new technique of microdialysis. They at first assumed that they were measuring the release from nerve terminals in the brain. However, there were accumulating discrepancies between the central release and peripheral release of the peptides, and when Morris & Pow (1991) showed that oxytocin and vasopressin could be released from all of the compartments of magnocellular neurons, not just the nerve terminals, Landgraf's student Ludwig (1998) realised that measurements of oxytocin and vasopressin in the magnocellular nuclei reflect the release of these neurons from the soma and dendrites, not from nerve terminals (Figs 2 and 3). Furthermore, he recognised that this dendritic release must somehow be regulated independently of terminal release.

This was a breakthrough – but how then was dendritic release regulated? Intriguing data from the laboratories of Theodosius and Hatton had indicated that in lactating animals, there was a morphological reorganisation of the supraoptic nucleus that might facilitate dendro-dendritic interactions: normally, the dendrites are separated from each other by interleaved glial cell processes, but in lactation, these processes are retracted,

**Figure 2**

(A) Vasopressin and oxytocin that circulate in the plasma are synthesised by magnocellular neurons whose cell bodies are located mainly in the paraventricular (PVN) and the supraoptic nuclei (SON) of the hypothalamus (vasopressin cells are immunostained with fluorescent green and oxytocin

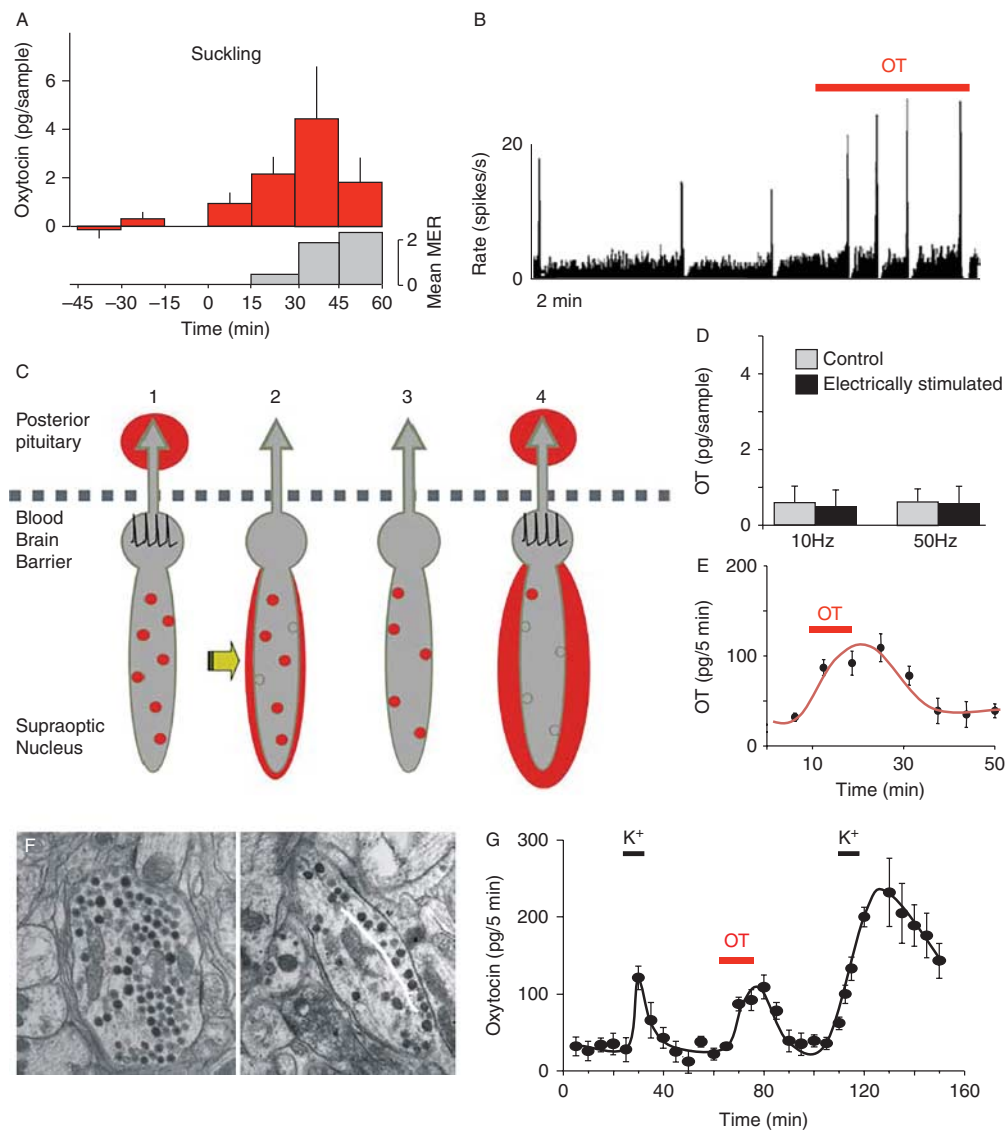
cells with fluorescent red). (B) The peptide immunostaining is punctate and represents individual or aggregates of large dense-cored vesicles, and in dendrites, the vesicles are particularly abundant.

which puts the dendrites of oxytocin neurons in direct apposition to each other within 'bundles' of dendrites (Hatton 1990, Theodosis & Poulain 1993). However, there was a stumbling block: oxytocin cells only show synchronous bursting during suckling and parturition – even during lactation, other stimuli increases their activity but never elicited bursts. Dyball & Leng (1986), working in Cross's group at the Babraham Institute, where Cross had become the director, pursued the idea that some kind of positive feedback was involved. They thought it possible that a recurrent excitatory circuit that involved interneurons was responsible – but although the intense stimulation of the neural stalk massively activated the cells in the supraoptic nucleus, it never triggered recurrent excitation in those cells. The stimulation was not without effect on the milk-ejection reflex, but the effects were quite subtle – there was a facilitation of bursting but only when stimuli were applied quite close to when a burst was expected to happen anyway.

Leng and Ludwig began to work together to address a basic question – would the intense electrical stimulation of the neural stalk actually release any vasopressin or oxytocin in the supraoptic nucleus? In experiment after experiment, the answer was frustratingly negative – there was no sign of release measured by microdialysis following electrical activation (Ludwig *et al.* 2002). Release could be evoked consistently by other kinds of stimulation, but without a link to the electrical activity of the cells, where was the positive feedback effect?

The next breakthrough came again from the Richard's lab, with the demonstration that oxytocin could cause a mobilisation of intracellular calcium stores in oxytocin cells (Lambert *et al.* 1994). How might that be relevant?

Working on the gonadotroph cells of the anterior pituitary gland, another of Harris's students, George Fink, had shown something remarkable. In oestrogen-primed rats, the secretion of luteinising hormone (LH) in response to gonadotrophin-releasing hormone (GnRH) increases

**Figure 3**

(A) Push–pull perfusion studies have shown that dendritic oxytocin release increases before the high-frequency burst activity of oxytocin neurons which is associated with the milk-ejection reflex. Reproduced, with permission, from Moos F, Poulain DA, Rodriguez F, Guerne Y, Vincent JD & Richard P (1989) Release of oxytocin within the supraoptic nucleus during the milk rejection reflex in rats. *Experimental Brain Research* 76 593–602. Copyright 1989 Springer-Verlag. (B) The i.c.v. injection of oxytocin increases the burst amplitude and the burst frequency of oxytocin cells, which shows that central release regulates the milk-ejection reflex. Reproduced, with permission, from Brown D, Fontanaud P & Moos FC (2000) The variability of basal action potential firing is positively correlated with bursting in hypothalamic oxytocin neurones. *Journal of Neuroendocrinology* 12 506–520. Copyright 2000 Blackwell Science Ltd. (C) Dendritic oxytocin release can be conditionally primed. Reproduced, with permission, from Ludwig M & Leng G (2006) Dendritic peptide release and peptide-dependent behaviours. *Nature Reviews Neuroscience* 7 126–136. Copyright 2006, Rights Managed by Nature Publishing Group. (D) Under normal conditions, dendritic peptide release is not activated by electrical (spike) activity. This is indicated by the lack of dendritic oxytocin release in response to electrical

stimulation of the neural stalk (light grey columns). (E) A conditional signal (arrow), such as oxytocin itself, triggers release from dendrites independently of the electrical activity. Reproduced, with permission, from Ludwig M, Sabatier N, Bull PM, Landgraf R, Dayanithi G & Leng G (2002) Intracellular calcium stores regulate activity-dependent neuropeptide release from dendrites. *Nature* 418 85–89. Copyright 2002, Rights Managed by Nature Publishing Group. (F) The conditional signal also primes dendritic stores. Priming occurs partially by the relocation of dendritic large dense-core vesicles closer to the dendritic plasma membrane. Reproduced, with permission, from Tobin VA, Hurst G, Norrie L, Dal Rio FP, Bull PM & Ludwig M (2004) Thapsigargin-induced mobilization of dendritic densecored vesicles in rat supraoptic neurons. *European Journal of Neuroscience* 19 2909–2912. Copyright 2004 Federation of European Neuroscience Societies. (G) After oxytocin-induced priming, the vesicles are available for activity-dependent release for a prolonged period. Reproduced, with permission, from Ludwig M, Sabatier N, Bull PM, Landgraf R, Dayanithi G & Leng G (2002) Intracellular calcium stores regulate activity-dependent neuropeptide release from dendrites. *Nature* 418 85–89. Copyright 2002, Rights Managed by Nature Publishing Group.

with successive exposures to GnRH, a phenomenon that Fink (1995) called 'self-priming'. With Morris and others, Fink showed that between exposures to GnRH, there is a 'margination' of secretory granules in gonadotrophs: how much LH is secreted in response to GnRH depends on how many granules lie close to the plasma membrane – and GnRH could trigger the relocation of granules to these sites (Lewis *et al.* 1986). This depends on the mobilisation by GnRH of intracellular calcium stores, so Leng and Ludwig, knowing that the release of neurosecretory granules in response to electrical activity was likely to depend on those granules being close to the site of depolarisation-induced calcium entry, wondered if something similar was happening in the dendrites of magnocellular neurons. By 'retrodialysis' – using microdialysis probes to deliver a substance rather than to collect one – they applied thapsigargin directly to the supraoptic nucleus to evoke a large increase in intracellular calcium in the magnocellular cells; then, long after the direct effects of the thapsigargin had worn off, they applied electrical stimulation to the neural stalk. Now, finally, they could see a dramatic electrically-evoked release of both oxytocin and vasopressin in the supraoptic nucleus as well as from the pituitary. They went on to show that the same 'priming' could be seen in response to peptides that evoked intracellular calcium mobilisation – including (for oxytocin release) oxytocin itself (Ludwig *et al.* 2002).

Rossoni *et al.* (2008) were then able to build a computational model of the oxytocin system that incorporated these phenomena and that reproduced the bursting behaviour of oxytocin neurons observed during the milk-ejection reflex. That model explained how bursts could be generated by dendro-dendritic intercommunication and could be rapidly propagated through the oxytocin cells in a hypothalamic nucleus, but it left unexplained how oxytocin cells in the two supraoptic and two paraventricular nuclei come to be activated simultaneously. One possibility lies in recognising that the appearance of the separation of the four nuclei is misleading – many magnocellular neurons are located between the main nuclear aggregations, some as small, 'accessory' nuclei and some as scattered neurons. Thus, if these neurons share dendro-dendritic contacts with the major aggregations, they might complete a network that links all of the nuclei. A second possibility arises from the work of Knobloch *et al.* (2012), who found that the paraventricular nucleus contains some non-neuroendocrine oxytocin neurons that innervate oxytocin cells in the supraoptic nucleus.

Parturition

Oxytocin's role in milk ejection is indispensable: animals that lack oxytocin are unable to feed their offspring (Nishimori *et al.* 1996, Young *et al.* 1996). By contrast, although oxytocin is named after its effects on uterine contractility, mice that lack oxytocin are still able to deliver young relatively normally, but whether this is generally the case in all mammals still remains unclear. In 1941, Ferguson reported that in the pregnant rabbit, distension of the uterus and cervix could induce the secretion of oxytocin, but in that same year, Dey *et al.* reported on the effects of lesions to the supraoptico-hypophysial tract in pregnant guinea pigs: of the 16 labours studied, ten were prolonged and difficult and ended in the death of the mother or the delivery of dead fetuses, but six were apparently normal (Dey *et al.* 1941, Ferguson 1941). Harris had shown that the electrical stimulation of the neural stalk could evoke strong uterine contractions, but it remained unclear whether the effects of oxytocin on the uterus reflected an active role of oxytocin in parturition or a pharmacological effect without real physiological significance (Harris 1948b). However, Harris's papers prompted Gunther (1948) to write a letter to the *BMJ*: she had observed labour in a woman who was still lactating after the birth of a previous child and noticed that beads of milk appeared at the nipples during each uterine contraction. Many factors were known to be capable of eliciting uterine contractions, but only oxytocin was known to induce milk let-down, so Gunther speculated that the uterine contractions provoked the release of oxytocin, which acted in a positive-feedback manner to support parturition.

However, by the end of the 1950s and the beginning of the 1960s, it was recognised that the plasma of pregnant women contained an enzyme – oxytocinase – that could potentially degrade oxytocin and that the levels of oxytocinase increased markedly towards term (Melander 1961). This greatly complicated measuring oxytocin in pregnancy and also raised fresh doubt about the physiological role of oxytocin: if oxytocin was important for parturition, it seemed to make no sense that the placenta should produce large amounts of an enzyme that destroyed it.

Then, in the 1980s, Summerlee and colleagues, working in Cross's former department at Bristol, published a series of papers that reported the activity of oxytocin neurons recorded over prolonged periods in conscious rats and rabbits through parturition and lactation (Summerlee 1981, Summerlee & Lincoln 1981, Paisley & Summerlee 1984, O'Byrne *et al.* 1986). These studies

achieved two things of particular importance: first, the milk-ejection reflex as described in anaesthetised rats was essentially identical to the reflex in conscious rats; and second, similar bursting activity was generated during parturition, linked to the delivery of young. The insight that oxytocin secretion is pulsatile during parturition cast a new light on the high levels of oxytocinase in the plasma of pregnant women, because although these high levels of oxytocinase diminish basal levels of oxytocin, they would also be expected to 'sharpen' the pulses of oxytocin by shortening their half-life. By frequent blood sampling combined with rigorous methods to inactivate oxytocinase in those samples, Fuchs *et al.* (1991) confirmed that spontaneous delivery in women is indeed associated with frequent short pulses of oxytocin secretion.

But are pulses necessary for parturition in the way that they are for milk ejection? This is less clear, because the uterus will continue to contract in the continued presence of oxytocin. Nevertheless, it seems that pulses are indeed a more effective way for oxytocin to drive parturition. At Babraham, Luckman *et al.* (1993) tested this in the rat by first interrupting parturition with morphine – a potent inhibitor of oxytocin neurons in the rat – and then attempting to re-establish parturition by giving oxytocin either as pulses or as a continuous infusion. Normal parturition could be reinstated by giving pulses of oxytocin at 10 min intervals, whereas much higher doses were needed to achieve a similar outcome by the continuous infusion of oxytocin.

It is now generally accepted that in all mammalian species, oxytocin secreted from the posterior pituitary plays a role in the expulsive phase of labour. Apart from its direct effects on the uterine myometrium, oxytocin also stimulates prostaglandin release by its actions on the decidua/uterine epithelium. Oxytocin is not strictly essential, seeing as other mechanisms can generally compensate for its absence, but it is secreted in very large amounts during labour; it also acts on the uterus, which expresses greatly increased levels of oxytocin receptor at term, by either acutely blocking oxytocin release or slowing parturition (Blanks & Thornton 2003, Russell *et al.* 2003). The trigger for initiating parturition varies between species, but it seems that oxytocin is commonly a driver for uterine contractions once parturition has begun (Russell *et al.* 2003, Arrowsmith & Wray 2014). Oxytocin may also play some part in the initiation of labour, but in women, other paracrine mechanisms are more important for this (Kamel 2010). However, oxytocin antagonists are used to avert threatened preterm labour (Usta *et al.* 2011).

Sexual activity

Harris (1947) showed that the stimulation of the posterior pituitary evoked robust uterine contractions in the oestrous or oestrogenised rabbit and that these effects could be mimicked by injections of pituitary extract. He knew that this did not demonstrate a physiological role for oxytocin in labour and that Ferguson's (1941) findings were more pertinent to that issue. However, he was intrigued that oxytocin caused uterine contractions in the empty, non-pregnant uterus, and he speculated that coitus might trigger the secretion of oxytocin to facilitate the transport of seminal fluid up the female reproductive tract. He went on to find a novel way of testing whether coitus triggered oxytocin secretion in women.

As described in the previous section, Gunther (1948) had reported the appearance of beads of milk in a lactating woman during labour, and this had impressed Harris as good evidence of the active secretion of oxytocin. In 1953, his colleague Pickles (1953) made a similar observation, this time of a lactating woman who had experienced milk let-down immediately after achieving orgasm. Together, Harris & Pickles (1953) set about to see if this was a common occurrence. Their approach was wonderfully direct – they asked the wives of their colleagues. Six had noticed milk let-down during some stage of coitus (not necessarily at orgasm), and two others reported the 'tingling experience' in their breasts that they recognised as being the same as the one they experienced during suckling. Because milk let-down is a reflex for which oxytocin is essential, this 'bioassay' was powerful evidence that oxytocin is indeed released during coitus in women, a conclusion that was later confirmed by RIA: there appears to be enhanced secretion in the arousal phase before orgasm (Carmichael *et al.* 1987), whereas the rises at orgasm itself are generally very small (Blancher *et al.* 1999).

Whether the secretion of oxytocin into blood during sexual activity has any physiological role in women is still unclear: Levin (2011) has argued that it plays little if any role in sperm transport. Oxytocin is also secreted into the blood during coitus in female goats (McNeilly & Ducker 1972), but there is an inconsistent increase in rabbits (Todd & Lightman 1986), and although oxytocin secretion in ewes increases in the presence of a ram, there is no further rise in secretion during mating itself (Gilbert *et al.* 1991). Large doses of oxytocin given systemically facilitate lordosis in ovariectomised, oestrogen-primed rats. Because central injections of much smaller amounts of oxytocin have a similar effect, it has been assumed that this effect is mainly a reflection of

actions within the brain. But because the effects of systemically administered oxytocin appear to depend on the presence of an intact uterus and cervix, peripheral actions may also contribute (Moody & Adler 1995).

In men, in response to masturbation, Murphy *et al.* (1987) found an increase in vasopressin secretion but not oxytocin secretion during sexual arousal and a large and robust increase in oxytocin secretion but not vasopressin secretion at ejaculation. Oxytocin and its receptors are expressed in the prostate, penis, epididymis, and testis, and there is good evidence that the peripheral actions of oxytocin support penile erection and ejaculation, and facilitate sperm transport (Corona *et al.* 2012).

Vasopressin secretion

Although Harris (1948c) showed that the electrical stimulation of the posterior pituitary in rabbits resulted in the appearance of a substance in the urine that had antidiuretic effects, this was not any great surprise. It was already clear that posterior pituitary extracts had marked antidiuretic effects, that the hormone content of the posterior pituitary was markedly depleted by dehydration, and that the urine of dehydrated animals contained a substance with apparently similar antidiuretic properties to those of posterior pituitary extracts. Verney (1947) had established that intracarotid infusions of hypertonic solutions elicited antidiuresis in dogs, and, through experiments that involved ligations of the internal carotid artery and various nerve sections, he had shown that this antidiuretic response required an intact posterior pituitary and that the osmoreceptors apparently lay in a region of the prosencephalon that is supplied by the internal carotid. The supraoptic nucleus itself was suggested to be a prime candidate for the location of these osmoreceptors, particularly because it was known to be exceptionally densely vascularised. Indeed, this speculation was correct – the magnocellular neurons of the supraoptic and paraventricular nuclei express stretch-sensitive membrane channels, which makes them exquisitely sensitive to volume change; with raised external osmolality, the cells shrink, and this shrinkage results in the activation of a depolarising current (Bourque 2008).

But this mechanism does not work in isolation. The direct depolarisation that results from volume changes is small and not enough in itself to increase the spiking activity of the magnocellular neurons. However, if those neurons are also receiving extensive afferent input, then even a small tonic depolarisation becomes effective, because it increases the probability that depolarisations

that arise from afferent input will exceed spike threshold. Thus, although the magnocellular neurons are osmoreceptors, when they are deafferented, they cannot increase their firing rates in response to osmotic stimulation – this response requires at least a tonic afferent input (Leng *et al.* 1982). They get such a tonic input from a set of anterior brain structures that includes two circumventricular organs – the subfornical organ and the organum vasculosum of the laminae terminalis – that are also osmoreceptive in the same way that magnocellular neurons are (Bourque 2008). They project to the magnocellular nuclei but also to the nucleus medianus, a midline structure adjacent to the anterior wall of the third ventricle which also projects densely to the magnocellular nuclei. Collectively, these anterior regions have become known as the ‘AV3V region’. This region controls not only antidiuresis but also thirst and natriuresis, and it mediates the effects of angiotensin produced by the kidney and those of other circulating hormones of cardiovascular origin (Johnson 1985).

Stress

Harris’s monograph focuses on another aspect of the regulation of vasopressin secretion that is more controversial – the effect of emotional stress. He noted that there was considerable evidence in humans that emotional stress was accompanied by antidiuresis, that Verney had shown this to also be the case in dogs, and that this seemed likely to be the result of vasopressin released from the posterior pituitary. In rats, many behavioural stressors have no clear effect on vasopressin secretion, although they generally do stimulate oxytocin secretion (Gibbs 1986), whereas conditioned fear stimulates oxytocin secretion but inhibits vasopressin secretion (Onaka *et al.* 1988), and novelty stress inhibits vasopressin secretion but has no effect on oxytocin secretion (Onaka *et al.* 2003). By contrast, in humans, vasopressin secretion appears to be stimulated by psychological stressors, such as social stress (Siegenthaler *et al.* 2014) and exam stress (Urwyler *et al.* 2015).

The physiological significance of this observation is very uncertain. Vasopressin plays an important role in regulating adrenocorticotrophic hormone (ACTH) secretion from the anterior pituitary; it is released into the hypothalamo-hypophysial portal circulation from the terminals of parvocellular and magnocellular neurons of the paraventricular nucleus and acts in concert with corticotrophin releasing factor (CRF) (Antoni *et al.* 1983). Circulating levels of vasopressin, which are secreted from

the posterior pituitary, are generally thought to be too low to be effective. However, vasopressin and CRF interact synergistically in stimulating ACTH secretion, so it is possible that in the presence of elevated CRF secretion, vasopressin secretion from the pituitary might become effective. To date, this possibility has not been extensively tested – and *Ehrenreich et al.* (1996) found no association in human subjects between increases in vasopressin secretion in response to novelty stress and ACTH secretion. Even if vasopressin from the magnocellular system does influence ACTH secretion under some circumstances, it is unclear what adaptive significance there might be. Similarly, the increased secretion of oxytocin in response to many stressors is without both clear physiological effect and adaptive significance. Oxytocin alone is an even weaker ACTH secretagogue than vasopressin is.

The present day

We now know that oxytocin and vasopressin have numerous peripheral targets that were largely or completely unknown to Harris. There is evidence that, in some species at least, oxytocin is involved in the regulation of natriuresis (*Antunes-Rodrigues et al.* 1997), osteoblast activity (*Di Benedetto et al.* 2014), and gastric motility (*Qin et al.* 2009).

However, the more radical change in our worldview has probably come from the recognition that oxytocin and vasopressin are not only secreted from the posterior pituitary but are also released in the brain, where they have very diverse behavioural effects. Both oxytocin and vasopressin are modulators of social behaviour (*Caldwell et al.* 2008, *Lee et al.* 2009, *Neumann & Landgraf* 2012). Parvocellular oxytocin and vasopressin neurons in the paraventricular nucleus project to many sites in the CNS and the spinal cord, and vasopressin is also expressed at several other sites in the brain (see *de Vries* 2008), including in the olfactory bulb, where it has been implicated in social recognition (*Tobin et al.* 2010). In addition, oxytocin is an important regulator of appetite (*Leng et al.* 2008) and sexual behaviour (*Baskerville & Douglas* 2008). Centrally projecting parvocellular oxytocin and vasopressin neurons play important roles in these behaviours, but the magnocellular neuroendocrine system has also been implicated through dendritic release mechanisms. It now seems clear that many neuroactive substances released in the brain, including oxytocin and vasopressin, can act at a distance from their sites of release (*Leng & Ludwig* 2008). Oxytocin and vasopressin have profound effects on behaviours that are exerted at sites

that, in some cases, richly express peptide receptors but are innervated by few peptide-containing projections. The release of these peptides is not specifically targeted at synapses, and the long half-life of peptides in the CNS and their abundance in the extracellular fluid mean that after release they can reach their sites of action by what *Fuxe* has called ‘volume transmission’ (*Fuxe et al.* 2012). At their targets, the process of priming allows peptides to functionally reorganise neuronal networks, which provides a substrate for prolonged behavioural effects (*Ludwig & Leng* 2006).

Our mechanistic understanding of the magnocellular neurons has undoubtedly achieved great sophistication (*Brown et al.* 2013), substantially through a concerted drive by many scientists over many years to meet the challenges laid down by Harris and his contemporaries – to understand the milk-ejection reflex, the role of oxytocin in parturition, and the nature of the osmoregulatory response of vasopressin cells. Discoveries of wide significance followed this drive, including: the recognition of the importance of pulsatile hormone secretion; the recognition of the importance of stimulus-secretion coupling mechanisms in interpreting the patterned electrical activity of neurons; the physiological importance of peptide release in the brain; the recognition that peptide release comes substantially from dendrites and can be regulated independently of nerve terminal secretion; and the importance of dynamic morphological changes to neuronal function in the hypothalamus. All of these discoveries followed directly from the drive to understand the milk-ejection reflex.

Yet despite the intensity with which magnocellular neurons have been interrogated, these neurons still have the capacity to surprise us. For example, it has only recently become clear that magnocellular vasopressin neurons are exquisitely thermosensitive (*Sudbury et al.* 2010) and are regulated by circadian inputs (*Trudel & Bourque* 2012).

In the present essay (we do not pretend it to be a comprehensive review), we seek to follow the impact of Harris’s work. Any such venture risks reinterpreting history to suit a narrative. Yet science is an inescapably social activity, and to neglect this fact would be a mistake. For good and bad, there are ‘bandwagons’ in our field, some of which crash in blind alleys, as we suspect will be the case for the current bandwagon of paying attention to the effects of the intranasal application of oxytocin and vasopressin, the behavioural consequences of which are generally ascribed, on little evidence, to central actions but which in our view are more likely to be incidental

consequences of peripheral actions. The bandwagons that Harris set rolling have, however, rolled and rolled, and they have led us inexorably to our present sophisticated and nuanced understanding of the magnocellular neurons.

Declaration of interest

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